AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims

Claims 1 – 30 (cancelled)

Claim 31 (withdrawn): Staphylokinase derivatives having essentially the amino acid sequence as depicted in figure 1 in which one or more amino acids have been replaced by another amino acid thus reducing the reactivity with a panel of murine monoclonal antibodies provided that the other amino acid is not alanine, wherein the staphylokinase derivatives are chemically modified with polyethylene glycol and are characterized by a significantly reduced plasma clearance.

Claim 32 (withdrawn): Staphylokinase derivatives as claimed in claim 31 having essentially the amino acid sequence as depicted in figure 1 in which one or more amino acids have been replaced by another amino acid thus reducing the absorption of SakSTAR-specific antibodies from plasma of patients treated with staphylokinase provided that the other amino acid is not alanine.

Claim 33 (withdrawn): Staphylokinase derivatives as claimed in claim 31 having essentially the amino acid sequence as depicted in figure 1 in which one or more amino acids have been replaced by other amino acids, without reducing the specific activity by more than 50 percent provided that the other amino acid is not alanine.

Claim 34 (withdrawn): Staphylokinase derivatives SakSTAR (K35X, G36X, E65X, K74X, E80X, D82X, K102X, E108X, K109X, K121X, K130X, K135X, K136X, +137X) having the amino acid sequence as depicted in figure 1 in which one or more of the amino

acids Lys in position 35, Gly in position 36, Glu in position 65, Lys in position 74, Glu in position 80, Asp in position 82, Lys in position 102, Glu in position 108, Lys in position 109, Lys in position 121, Lys in position 130, Lys in position 135 and/or Lys in position 136 have been replaced with other amino acids provided that the other amino acid is not alanine and/or in which one amino acid has been added at the COOH-terminus, thus altering the immunogenicity after administration in patients, without markedly reducing the specific activity, wherein the staphylokinase derivatives are chemically modified with polyethylene glycol and are characterized by a significantly reduced plasma clearance.

Claim 35 (withdrawn): Staphylokinase derivatives listed in Tables 1, 3, 4, 5, 6, 7, 8, 13, 19, and 20, having the amino acid sequence as depicted in figure 1 in which the indicated amino acids have been replaced by other amino acids thus reducing the absorption of SakSTAR-specific antibodies from plasma of patients treated with staphylokinase, without reducing the specific activity, provided that at least one amino acid is replaced with an amino acid other than alanine, wherein the staphylokinase derivatives are chemically modified with polyethylene glycol and are characterized by a significantly reduced plasma clearance.

Claim 36 (withdrawn): Staphylokinase derivative as claimed in claim 31, selected from the group consisting of SakSTAR (S34G, G36R, H43R), SakSTAR (S34G, G36R, H43R), SakSTAR (G36R), SakSTAR (H43R), SakSTAR (G36R, K74R), SakSTAR (K35E), SakSTAR (K74Q), SakSTAR (K130T), SakSTAR (V132L), SakSTAR (V132T), SakSTAR (V132N), SakSTAR (V132R), SakSTAR (K130T, K135R), SakSTAR (G36R, K130T, K135R), SakSTAR (K74R, K130T, K135R), SakSTAR (K74Q, K130T, K135R), SakSTAR (G36R, K74R, K130T, K135R), SakSTAR (G36R, K74Q, K130T, K135R), SakSTAR (G36R, H43R, K74R, K130T, K135R), SakSTAR (E65A, K74Q, K130T, K135R), SakSTAR

(E65Q, K74Q, K130T, K135R), SakSTAR (K74Q, K86A, K130T, K135R), SakSTAR (E65Q, T71S, K74Q, K130T, K135R), SakSTAR (K74Q, K130A, K135R), SakSTAR (E65Q, K74Q, K130A, K135R), SakSTAR (K74Q, K130E, K135R), SakSTAR (K74Q, K130E, V132R, K135R), SakSTAR (E65Q, K74Q, T90A, K130A, K135R), SakSTAR (E65Q, K74Q, N95A, K130A, K135R), SakSTAR (E65Q, K74Q, E118A, K130A, K135R), SakSTAR (E65Q, K74Q, N95A, E118A, K130A, K135R), SakSTAR (N95A, K130A, K135R), SakSTAR (E65Q, K74Q, K109A, K130T, K135R), SakSTAR (E65Q, K74Q, E108A, K109A, K130T, K135R), SakSTAR (E65Q, K74Q, K121A, K130T, K135R), SakSTAR (E65Q, K74Q, N95A, E118A, K130A, K135R, K136A, +137K), SakSTAR (E80A, D82A, K130T, K135R), SakSTAR (K74R, E80A, D82A, K130T, K135R), SakSTAR (K74Q, E80A, D82A, K130T, K135R), SakSTAR (K35A, K74R, E80A, D82A, K130T, K135R), SakSTAR (E65D, K74R, E80A, D82A, K130T, K135R), SakSTAR (E65S, K74R, E80A, D82A, K130T, K135R), SakSTAR (S34G, G36R, K74R, K130T, K135R), SakSTAR (E65A, K74R, E80A, D82A, K130T, K135R), SakSTAR (E65N, K74R, E80A, D82A, K130T, K135R), SakSTAR (E65Q, K74R, E80A, D82A, K130T, K135R), SakSTAR (K57A, E58A, E61A, E80A, D82A, K130T, K135R), SakSTAR (E65D, K74Q, E80A, D82A, K130T, K135R), SakSTAR (E65Q, K74Q, E80A, D82A, K130T, K135R), SakSTAR (K35A, E65D, K74Q, E80A, D82A, K130T, K135R), SakSTAR (K74R, E80A, D82A, S103A, K130T, K135R), SakSTAR (E65D, K74R, E80A, D82A, K109A, K130T, K135R), SakSTAR (E65D, K74R, E80A, D82A, K130T, K135R, K136A), SakSTAR (E65Q, K74Q, D82A, S84A, K130T, K135R), SakSTAR (K35A, K74Q, E80A, D82A, K130T, K135R), and SakSTAR (K35A, E65D, K74R, E80A, D82A, K130T, K135R).

Claim 37 (withdrawn): SakSTAR (E65D, K74R, E80A, D82A, K130T, K135R) having the code SY19 which is chemically modified with polyethylene glycol and is characterized by a significantly reduced plasma clearance.

Claim 38 (withdrawn): SakSTAR (K35A, E65Q, K74R, E80A, D82A, T90A, E99D, T101S, E108A, K109A, K130T, K135R) having the code SY161 which is chemically modified with polyethylene glycol and is characterized by a significantly reduced plasma clearance.

Claim 39 (currently amended): A staphylokinase derivative comprising ~~having essentially the~~ an amino acid sequence which differs from ~~as depicted in figure 1 (~~SEQ ID NO: 1~~)~~ due to substitution of at least one of amino acids therein with another amino acid, wherein the at least one amino acid is substituted with Cys, wherein the substitution allows the formation of a homodimeric form of the staphylokinase derivative through the formation of an intermolecular disulfide bridge, wherein the staphylokinase derivative has a reduced reactivity with a panel of murine monoclonal antibodies having specific reactivity with ~~towards~~ staphylokinase, ~~and having in addition either one or both of the following:~~

~~(a) at least one amino acid substituted with Cys, wherein the substitution is introduced at a position outside both the binding epitope and activation epitope of the staphylokinase molecule, and wherein the substitution allows the formation of a homodimeric form of staphylokinase through the formation of an intermolecular disulfide bridge; and/or~~

~~(b) polyethylene glycol coupling to an amino acid residue, wherein the coupling is introduced at a position outside both the binding epitope and the activation epitope, resulting in a significantly reduced plasma clearance while maintaining specific activity.~~

Claim 40 (currently amended): The ~~S~~staphylokinase derivative as claimed in claim 39, ~~having essentially the amino acid sequence as depicted in figure 1 (SEQ ID NO: 1) in which one or more amino acids have been replaced by another amino acid thus~~ wherein the staphylokinase derivatives have a ~~reducing~~ reduced ~~the~~ absorption of SakSTAR (a specific wild-type variant of staphylokinase)-specific antibodies from plasma of patients treated with staphylokinase.

Claim 41 (currently amended): ~~Staphylokinase derivatives~~ The staphylokinase derivative as claimed in claim 39, ~~having essentially the amino acid sequence as depicted in figure 1 in which one or more amino acids have been replaced by other amino acids~~ wherein the specific activity of said derivatives is at least 50% that of the corresponding wild-type staphylokinase.

Claim 42 (withdrawn): Staphyokinase derivatives as claimed in claim 39, named SakSTAR (K35X, G36X, E65X, K74X, E80X, D82X, K102X, E108X, K109X, K121X, K130X, K135X, K136X, +137X) and having the amino acid sequence as depicted in figure 1 in which one or more of the amino acids Lys in position 35, Gly in position 36, Glu in position 65, Lys in position 74, Glu in position 80, Asp in position 82, Lys in position 102, Glu in position 108, Lys in position 109, Lys in position 121, Lys in position 130, Lys in position 135 and/or Lys in position 136 have been replaced with other amino acids and/or in which one amino acid has been added at the COOH-terminus, thus altering the immunogenicity after administration in patients, without markedly reducing the specific activity.

Claim 43 (currently amended): The ~~S~~staphylokinase derivative as claimed in claim 39, ~~and listed in Tables 1, 3, 4, 5, 6, 7, 8, 13, 19 and 20, having the amino acid sequence as~~

~~depicted in figure 1 (SEQ ID NO: 1) thus reducing the~~ having a reduced absorption of SakSTAR-specific antibodies from plasma of patients treated with staphylokinase, without reducing the specific activity.

Claim 44 (withdrawn): Staphylokinase derivative as claimed in claim 39, selected from the group consisting of SakSTAR (K74A, E75A, R77A), SakSTAR (K35A, E75A), SakSTAR (E75A), SakSTAR (E80A, D82A), SakSTAR (E80A), SakSTAR (D82A), SakSTAR (E75A, D82A), SakSTAR (S34G, G36R, H43R), SakSTAR (K35A), SakSTAR (D82A), SakSTAR (D82A, S84A), SakSTAR (T90A), SakSTAR (Y92A), SakSTAR (K130A), SakSTAR (V132A), SakSTAR (S34G, G36R, H43R), SakSTAR (G36R), SakSTAR (H43R), SakSTAR (G36R, K74R), SakSTAR (K35E), SakSTAR (K74Q), SakSTAR (K130T), SakSTAR (V132L), SakSTAR (V132T), SakSTAR (V132N), SakSTAR (V132R), SakSTAR (K130T, K135R), SakSTAR (G36R, K130T, K135R), SakSTAR (K74R, K130T, K135R), SakSTAR (K74Q, K130T, K135R), SakSTAR (G36R, K74R, K130T, K135R), SakSTAR (G36R, K74Q, K130T, K135R), SakSTAR (G36R, H43R, K74R, K130T, K135R), SakSTAR (E65A, K74Q, K130T, K135R), SakSTAR (E65Q, K74Q, K130T, K135R), SakSTAR (K74Q, K86A, K130T, K135R), SakSTAR (E65Q, T71S, K74Q, K130T, K135R), SakSTAR (K74Q, K130A, K135R), SakSTAR (E65Q, K74Q, K130A, K135R), SakSTAR (K74Q, K130E, K135R), SakSTAR (K74Q, K130E, V132R, K135R), SakSTAR (E65Q, K74Q, T90A, K130A, K135R), SakSTAR (E65Q, K74Q, N95A, K130A, K135R), SakSTAR (E65Q, K74Q, E118A, K130A, K135R), SakSTAR (E65Q, K74Q, N95A, E118A, K130A, K135R), SakSTAR (N95A, K130A, K135R), SakSTAR (E65Q, K74Q, K109A, K130T, K135R), SakSTAR (E65Q, K74Q, E108A, K109A, K130T, K135R), SakSTAR (E65Q, K74Q, K121A, K130T, K135R), SakSTAR (E65Q, K74Q, N95A, E118A,

K130A, K135R, K136A, +137K), SakSTAR (E80A, D82A, K130T, K135R), SakSTAR (K74R, E80A, D82A, K130T, K135R), SakSTAR (K74Q, E80A, D82A, K130T, K135R), SakSTAR (K35A, K74R, E80A, D82A, K130T, K135R), SakSTAR (E65D, K74R, E80A, D82A, K130T, K135R), SakSTAR (E65S, K74R, E80A, D82A, K130T, K135R), SakSTAR (S34G, G36R, K74R, K130T, K135R), SakSTAR (E65A, K74R, E80A, D82A, K130T, K135R), SakSTAR (E65N, K74R, E80A, D82A, K130T, K135R), SakSTAR (E65Q, K74R, E80A, D82A, K130T, K135R), SakSTAR (K57A, E58A, E61A, E80A, D82A, K130T, K135R), SakSTAR (E65D, K74Q, E80A, D82A, K130T, K135R), SakSTAR (E65Q, K74Q, E80A, D82A, K130T, K135R), SakSTAR (K35A, E65D, K74Q, E80A, D82A, K130T, K135R), SakSTAR (K74R, E80A, D82A, S103A, K130T, K135R), SakSTAR (E65D, K74R, E80A, D82A, K109A, K130T, K135R), SakSTAR (E65D, K74R, E80A, D82A, K130T, K135R, K136A), SakSTAR (E65Q, K74Q, D82A, S84A, K130T, K135R), SakSTAR (K35A, K74Q, E80A, D82A, K130T, K135R), and SakSTAR (K35A, E65D, K74R, E80A, D82A, K130T, K135R).

Claim 45 (currently amended): The Sstaphylokinase derivative as claimed in claim 39, wherein the Cys ~~is chemically modified with~~ has coupled thereto polyethylene glycol, wherein the polyethylene glycol can have a molecular weight of up to 20 kDa.

Claim 46 (currently amended): The staphylokinase derivative of claim 45, wherein selected amino acids in the NH_2-terminal region of 10 amino acids (SEQ ID NO: 1 positions 1-10) are substituted with Cys, which ~~is chemically modified with~~ has coupled thereto polyethylene glycol, and wherein the derivatives are characterized by a significantly reduced plasma clearance and maintained thrombolytic potency upon single intravenous bolus administration at a reduced dose.

Claim 47 (currently amended): The ~~S~~staphylokinase derivative as claimed in claim 46, wherein the Ser in position 2 or 3 (SEQ ID NO: 1) is substituted with a Cys and the Cys ~~is chemically modified with~~ has coupled thereto polyethylene glycol having a molecular weight ~~of 5, 10 or 20~~ of up to 20 kDa.

Claim 48 (withdrawn): Staphylokinase derivative as claimed in claim 47, which derivative is SakSTAR (S3C-MP5, K35A, E65Q, K74R, E80A, D82A, T90A, E99D, T101S, E108A, K109A, K130T, K135R) having the code of SY161 (S3C-MP5).

Claim 49 (withdrawn): Staphylokinase derivative as claimed in claim 47, which derivative is SakSTAR (S3C-P10, K35A, E65Q, K74R, E80A, D82A, T90A, E99D, T101S, E108A, K109A, K130T, K135R) having the code of SY161 (S3C-P10).

Claim 50 (withdrawn): Staphylokinase derivative as claimed in claim 47, which derivative is SakSTAR (S3C-P20, K35A, E65Q, K74R, E80A, D82A, T90A, E99D, T101S, E108A, K109A, K130T, K135R) having the code of SY161 (S3C-P20).

Claim 51 (previously presented): The staphylokinase derivative of claim 47 labeled SY19 (S3C-MP5), wherein the derivative comprises amino acid substitutions S3C-MP5, E65D, K74R, E80A, D82A, K130T, K135R and wherein the cysteine at position 3 is chemically modified with MAL-PEG 5 kDa.

Claim 52 (withdrawn): Staphylokinase derivative as claimed in claim 47, which derivative is SakStar(S3C-SP5, E65D, K74R, E80A, D82A, K130T, K135R) having the code of SY19 (S3C-SP5).

Claim 53 (withdrawn): Staphylokinase derivative as claimed in claim 47, which derivative is SakSTAR (S2C-SP5, S3C-SP5, E65D, K74R, E80A, D82A, K130T, K135R) having the code of SY19 (S2C-SP5, S3C-SP5).

Claim 54 (withdrawn): Staphylokinase derivative as claimed in claim 47, which derivative is SakSTAR (S3C-P20, E65D, K74R, E80A, D82A, K130T, K135R) having the code of SY19(S3C-P20).

Claim 55 (withdrawn): Staphylokinase derivative as claimed in claim 47, which derivative is SakSTAR (S3C-P20, E65D, K74R, E80A, D82A, K130T, K135R) having the code of SY19(S3C-P10).

Claim 56 (withdrawn): Dimer of two staphylokinase derivatives as claimed in claim 39.

Claim 57 (withdrawn): Method for producing the staphylokinase derivatives as claimed in claim 31, comprising the steps of:

preparing a DNA fragment comprising at least the part of the coding sequence of staphylokinase that provides for its biological activity;

performing *in vitro* site-directed mutagnesis on the DNA fragment to replace one or more codons for wild-type amino acids by a codon for another amino acid;

cloning the mutated DNA fragment in a suitable vector;

transforming or transfecting a suitable host cell with the vector; and

culturing the host cell under conditions suitable for expressing the DNA fragment.

Claim 58 (withdrawn): Method as claimed in claim 57, wherein the DNA fragment is a 453 bp Eco-RI-HindIII fragment of the plasmid pMEX602sakB, the *in vitro* site-directed mutagenesis is performed and the mutated DNA fragment is expressed in *E. Coli*.

Claim 59 (withdrawn): Pharmaceutical composition comprising at least one of the staphylokinase derivatives as claimed in claim 31, together with a suitable excipient.

Claim 60 (withdrawn): Pharmaceutical composition as claimed in claim 59 for treating arterial thrombosis.

Claim 61 (currently amended): The staphylokinase derivative of claim 39, wherein the at least one amino acid substituted with Cys is ~~at least one~~ selected from the group consisting of a surface exposed residue, a charged residue, a threonine residue and a serine residue.

Claim 62 (currently amended): The staphylokinase derivative of claim 39, wherein the at least one amino acid substituted with Cys ~~is the position of the~~ has coupled thereto polyethylene glycol ~~coupling~~.

Claim 63 (new): A staphylokinase derivative comprising an amino acid sequence which differs from SEQ ID NO: 1 due to substitution of at least one amino acid therein with another amino acid, having a reduced reactivity with a panel of murine monoclonal antibodies having specific reactivity with staphylokinase and having a polyethylene glycol coupling to an amino acid residue, wherein the coupling is introduced at a position outside both the binding epitope and the activation epitope, resulting in significantly reduced plasma clearance while maintaining staphylokinase specific activity.

Claim 64 (new): The staphylokinase derivative of claim 63, wherein the at least one amino acid is substituted with cysteine.

Claim 65 (new): The staphylokinase derivative of claim 64, wherein the polyethylene glycol coupling occurs at the substituted cysteine.

Claim 66 (new): The staphylokinase derivative of claim 64, wherein the at least one amino acid substituted with Cys is selected from the group consisting of a surface exposed residue, a charged residue, a threonine residue and a serine residue.

F1 Cont

Claim 67 (new): The staphylokinase derivative of claim 64, wherein selected amino acids in the NH_2-terminal region of 10 amino acids (SEQ ID NO: 1 positions 1-10), are substituted with Cysteine, which has attached thereto polyethylene glycol, said staphylokinase derivatives are characterized by a significantly reduced plasma clearance and maintained thrombolytic potency.